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Influence of Phosphorus in Feeds on the Phosphorus Content of Egg, and the Chemical Character of the Phosphorus Compounds

A

THESIS

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL

OF

CORNELL UNIVERSITY

FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY

BY

ARTHUR JOHN WILSON

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The study of the metabolism of feeding stuffs has not been extended equally to all classes of animals. The larger bulk of available data has been on the herbivora, but little to the chicken which may be called the link between the carnivorous and herbivorous. The structure of the alimentary canal of the bird suggests that the digestion process is rapid and partakes of features of both the carnivorous and herbivorous.

Phosphorus is one of the most interesing of the mineral elements found in animal because of the essential connections which it sustains with many of the structures and processes. It exists in the body in many compounds belonging to at least four groups.

1. Inorganic phosphates which occur throughout the body in solid and liquid state.
2. Lecithin found in all parts of plant and animal cells.
3. Phospho-proteids.
4. Nuclea-proteids.

The last two classes are probably constant cell-components.

In the inorganic phosphates phosphorus is present as salts of the mineral bases Ca, Mg, Na, K, and Fe. The lecithin phosphorus is present as glyco-phosphoric acid.

Phospho-proteins, to which class casein containing phosphorus belongs, the condition of the phosphorus is not positively known.

The phosphorus of the nucleo-proteins occurs mostly in the cell nucleins, and is present in the nuclic acid.

Care must be exercised in the choice of foods. There must be an abundant supply of mineral nutrients, especially is this so of the chicken. The specific effect of foods is partly due to relative proportion of carbohydrates, proteids and fats, the mineral nutrients are also in part too. It is a well known fact that if corn is fed excessively it will injure the egg-laying capacity of fowls. This is due to the tendency of the fat to accumulate internally in the

vicinity of the female generating organs, causing pressure, thus restricting the flow of blood. What corn, as a food lacks, must be provided for in some other form.

The organic phosphorus as it occurs in the egg is chiefly in the form of lecithin. This compound is sometimes prescribed as medicine in cases of rickets and nervous exhaustion. In the digestion process of this compound phosphorus is absorbed as glycero-phosphoric acid. The function of lecithin is not understood. The opinion of many experimenters in physiological chemistry is that the phosphorus of lecithin has a value as a nutrient that it has not in other compounds as a stimulent to growth. Inorganic phosphates are easily digested and assimilated. There is a difference of opinion as to whether the inorganic phosphates are useful in the building up of organic phosphorus compound in animal body.

It has been shown that bone meal and bone flour fed to pigs had the effect of strengthening the bones[1].

According to Bulletin of Ohio[2] the principal need of phosphorus in the body is for inorganic phosphorus; organic phosphorus can supply the bodily needs for phosphorus, both organic and inorganic, provided the necessary bases are present.

Purpose

To study the effect of feed stuffs, those rations that have a high organic phosphorus content versus rations having low organic content.

To determine if possible whether the phosphorus in the inorganic form is metabolized or not.

The effect of a feed high in organic phosphorus coupled with a grit low in inorganic phosphorus.

A feed low in organic phosphorus coupled with a grit high in inorganic phosphorus.

The nutritive plan of the ratio to be maintained the same.

Experimental Method

The feed to be carefully analyzed for organic and inorganic phosphorus.

The eggs to be analyzed both before and during the feeding, at uniform periods for organic and inorganic phosphorus.

1. Bull. 81, Mo. Agr. Exp. Station.
2. Bull. 201, Ohio Agr. Exp. Station.

Four pens containing five s. c. white leghorn pullets each.

All stock to be of uniform age and condition and about six months old at the beginning of the experiment.

Organic ration:

The ration employed—1 lb. buckwheat (whole), 1 lb. wheat (untreated), 1-2 lb. pea meal, oyster shell, micro-spar, cubical grit provided. Nutritive ratio 1:5.6.

Inorganic ration:

1 lb. corn, 1 lb. oats, 1 lb. wheat (treated), 1 lb. ground bone. The bone meal to be fed in a quantity equal to the consumption of oyster shell in first pen. Microspar cubical grit. Nutritive ratio 1:5.4.

The fowls to be fed the grain rations as much as they will consume and no more to be fed until all has been eaten up. The grain is scattered among the litter on the floor.

The remaining feeds are kept in separate hoppers and weighed at intervals to calculate amount consumed.

The wheat to be treated with water for twenty-four hours, rinsed and drained one-half hour, then fed; or a quantity treated thus, and dried to prevent fermentation; samples analyzed before and after treatment for organic and inorganic phosphorus.

The eggs collected daily, yolks and whites dried and analyzed collectively for organic and inorganic phosphorus.

The fowls to be fed on light diet for several weeks previous to the rations quoted in order to get them as uniform as possible.

The eggs to be grouped in series of three or four day periods in order to get enough eggs for the analyses and have comparable results.

The shells collected and analyzed for total phosphorus in similar groups.

The method of determining the total phosphorus used in the analysis is a modification of the Neuman method[1]. Concentrated nitric acid was used in the place of ammonium nitrate and a small piece of copper wire was added as it aids in the oxidation and also an indicator when neutralizing with ammonium hydroxide.

1. DuBois, Reymonds Archiv (Physion. Abth.) 1897, p. 552-553.

Neuman method—5 grams of material placed in a Kjeldahl flask, 10-15 cc. of concentrated sulphuric acid added, and the mixture heated over a low flame until well charred, when partly cool add 5 to 10 grams of ammonium nitrate and continue heating and adding ammonium nitrate until the mixture has been completely oxidized and decolorized. In the method as used concentrated nitric acid was used to replace the ammonium nitrate and small amounts added at a time until complete oxidation has taken place. On cooling the solution was transferred to a 500 cc. graduated flash and made up to the mark. An aliquot was taken for the determination of total phosphorus by the molybdate; magnesia added.

Separation of Inorganic from Organic Phosphorus

The various methods as given for the separation of inorganic from organic phosphorus in plant and animal tissue, the following seems most probable as a staring basis. Iwanow[1] separated as follows, 5 to 7 grams of the material was warmed on a water bath ten or fifteen minutes with 100 to 150 cc, of 1 per cent. acetic acid. After cooling, the precipitated proteid matter was separated by filtering, and washing with water until about 500 cc. filtrate had been collected for the determination of inorganic phosphorus. An aliquot from the 500 cc. was taken and precipitated directly with molybdate solution.

Zaleski[1] worked on the same problem but independently evolved an almost identical method. Zaleski recommends the use of either a 1 per cent. acetic acid solution or a .2 per cent. hydrochloric acid solution as the extracting reagent. The cleavage action, as shown later, was not sufficiently taken into account on the nucleins by the nitric acid contained in the ammonium molybdate. This author recommended that more nitric acid be added although he did not use it himself.

Kossel[2]. The author worked on muscle extracts and used a mixture of 5 per cent. hydrochloric acid and tannin as a precipitant of the proteids containing phosphorus.

Araki[3] has recently used this method somewhat modified in his studies of nucleic acid by enzymes. The method is as follows: 2 grame of a salt of nucleic acid dissolved in 40 cc. of water and to

1. Ber. deut. bot. Gesell., *20*, 336 (1902).
1. Ber. deut. bot. Gesell., *20*, 426 1902).
2. Zeits. Physiol.-Chem., 7, 9 (1882).
3. Zeits. Physiol.-Chem., *36*, 84 (1903).

this the enzyme was added. This solution was diluted with an equal volume of water, 4 grains of sodium acetate added and tannin so long as a precipitate continues to be formed. The filtrate, according to the author of this method, should contain any phosphoric acid split off by the action of enzymes. According to later investigations, tannic acid in dilute mineral acid solution and tannic acid in sodium acetate both failed to precipitate completely the soluble nucleins contained in certain of the feeding material.

The Hart-Andrews[1] method is superior to those just mentioned yet it is not in itself reliable. The method briefly is as follows: Extract 5 grams of material for fifteen minutes in 125 cc. of 2 per cent hydrochloric acid, filter, wash with water until the filtrate is 500 cc., neutralize 200 cc. of this with ammonium hydroxide precipitate at 65 degrees with 10 grains of ammonium nitrate and 25 cc. of neutral ammonium molybdate solution and 2 cc. of nitric acid, Sp. Gr. 1.20. Keep at this temperature fifteen minutes, allow to cool and filter after one hour. Treat this precipitate in the usual manner. Dissolve in ammonia. Precipitate with magnesium mixture and burn to the pyrophosphate, then redissolve and reprecipitate. The neutral ammonium molybdate is used to reduce the decomposition of nucleic acid to a minimum.

The method to be used in this study is the one employed by the Ohio Experiment Station[2]. This method which has been brought forward recently seems to be more accurate, since by investigation they have found reason to question the Hart-Andrews method in the following:

1. Time allowed for extraction appears to be insufficient.
2. The precipitate with neutral ammonium molybdate solution and the minimum amount of acid is rendered difficult as a routine method by the fact that 2 cc. of 1.20 nitric acid are in many cases not nearly enough to cause precipitation.
3. The bulky flocculent precipitate which often formed may frequently mask the yellow precipitate of inorganic phosphorus rendering it impossible to tell when the precipitation is complete.
4. The precipitate is difficult to filter.
5. The method involves the precipitating of inorganic phosphorus in the presence of Phytin, a quantitative precipitation of inorganic phosphorus alone from a solution containing Phytin appears to be impossible.
6. Nucleic acid may be hydrolyzed by the nitric acid used in

1. Bull. 238, N. Y. Agr. Exp. Station.
2. Bull. 2*5, Ohio Exp. Station.

the precipitation, resulting in the formation of inorganic phosphorus.

8. Possibility of error lies in the carrying down of phosphorus containing proteids with the yellow precipitate. The solution of these proteids in ammonia and then precipitating with magnesium mixture and the increasing of the final weight.

The method for the separation of organic from inorganic as given in the Ohio Bulletin for Plant Substances is as follows:

Pour exactly 300 cc. of .2 per cent. hydrochloric acid on 10 grams of finely ground sample in a 400 cc. flask, close with a rubber stopper and shake at intervals of five minutes for three hours. Filter the extract through dry filters into dry flasks (this filtration may have to be carried on by gentle suction, using a platinum cone to prevent rupture of the paper). Measure out a 250 cc. portion of this filtrate, precipitate in a 400 cc. beaker with 15 cc. of magnesium mixture and 300 cc. of strong ammonia, allow to stand over night and filter through a double 11 cm. S. & S. No. 595 filter, taking care to decant as long as possible without pouring off the precipitate. Then complete the transfer of the precipitate to the paper. Suction may also help this filtration toward the end, using a platinum cone.

The precipitate consists of magnesium ammonium phosphate with Phytin, when this compound is present in the substance involved and small quantities of variously colored unknowns, free nucleic acid and some of their salts are soluble in ammonia and these are gotten rid of. Wash several times with 2.5 per cent. ammonia and then several times with 95 per cent. alcohol until free from ammonia. The alcohol clears up the precipitate by dissolving out a large part of the colored compounds not effecting the Phytin and magnesium ammonium phosphate. The precipitate is allowed to drain, and then spread out on the inner paper, allowing the alcohol to evaporate. When practically dry, place the paper containing the precipitate in an Erlenmeyer flask, add 100 cc. of 95 per cent. alcohol containing .2 per cent nitric acid. Close the flask with a rubber stopper and shake until the paper is thoroughly broken up; if the precipitate is flaky and refuses to break up on shaking allow to stand over night in acid alcohol. This .2 per cent. nitric acid dissolves out the inorganic phosphorus but does not affect the Phytin. Now filter through a dry filter into a dry flask, pipette out 75 cc. of the filtrate into a small beaker, evaporate almost to dryness, then determine the phosphorus in the usual gravimetric

way by first precipitating with ammonium molybdate, then with magnesium mixture and burn to the pyrophosphate and weigh.

It was found necessary to modify this method when applied to the separation of the organic from the inorganic phosphorus in the egg. As lecithin is the principal form in which the organic phosphorus occurs it was though best to study this method when applied to a mixture containing the phosphorus in this form.

Preparation of Lecithin

The various authors advise the preparation and purification of lecithin by extracting the egg with absolute alcohol or ether or both separately. Evaporate the extractions to dryness and take up with as little ether as possible; filter and treat with about twice its volume of acetone. The acetone precipitates out the lecithin. Others use a chloroform solution and acetone as the precipitating agent. Neither of these methods work satisfactorily on the dried eggs as obained from the factory. The following method was used with very good results. The egg extracted with chloroform, the chloroform expelled and the residue dried in a water oven until a thick reddish brown mass was obtained. This mass was dissolved in warm ethyl acetate, when cooled down by means of ice the lecithin separates out and may be filtered off by using a funnel jacketed with ice. The lecithin is obtained on the filter. By repeating this treatment nearly pure lecithin may be obtained. A sample thus obtained was dried and on analysis gave 9.16 per cent. P_2O_5[1]. The amount of phosphorus anhydride varies from 8.75 to 9.45 per cent. P_2O_5. The sample was of a waxy appearance and of a reddish brown color.

The first thing to be studied is whether the lecithin or various compounds of the egg are broken up yielding phosphorus that will be precipitated with ammonium moylbdate solution when treated with the .2 per cent. HCl extracting reagent.

The lecithin purified by the ethyl acetate method was digested by shaking at frequent intervals for three hours. To this extract after filtering ammonium nitrate was added and neutral ammonium molybdate, digested one hour at 60 degrees. There was no yellow ammonium phosphomolybdate precipitated.

Taking the residue remaining after the extraction of the lecithin with ethyl acetate this residue contains chiefly fats and coloring

1. Imbert and Merle (Bull. de Pharm. Sud. Ext. May, 1902), Am. de Chim. Anel., 1902, 350-351.

matter. Treating this with the neutral ammonium molybdate in like manner and allowing to stand there was no precipitate formed.

The egg residue from the extraction with chloroform was treated with the .2 per cent. hydrochloric acid solution on the addition of the ammonium nitrate there was a flocculent precipitate thrown down, this could be cleared up with the addition of strong nitric acid, but on neutralizing this acid and precipitating with the ammonium molybdate there was a small precipitate obtained most of it being the protein bodies thrown down along with a small amount of inorganic phosphorus.

It seems to be safe to say that the .2 per cent. hydrochloric acid solution when allowed to act on the various portions will not break down the organic phosphorus compounds and yield phosphorus that will be precipitated as the inorganic.

The next question was to determine whether the magnesium mixture with the 12 cc. of ammoninum hyroxide, Sp. Gr. 0.90, would have any effect upon the hydrochloric acid extract, as this forms with the lecithin an emulsion, a semi-soluble mixture that goes through the filter paper. Taking the lecithin and allowing to stand with the magnesium mixture and 12 cc. of ammonium hydroxide over night there was a ligh flocculent precipitate formed. This did not have the appearance of pure magnesium phosphate. When the residue left after extracting the lecithin is treated in like manner there was no precipitate formed. The residue of the egg after extracting with chloroform was treated similarly and a faint precipitate appeared. On treating them as before but omitting the 12 cc. of ammonium hydroxide, Sp. Gr. .90, there was no precipitate formed where the lecithin gave one before. The slight precipitate caused by using the strong ammonia in the case of the lecithin is probably caused by saponification of the lecithin, as lecithin yields on saponification fatty acids, phosphoric acid and glycerol, (cholin)[1].

In order to find whether or not the .2 per cent. hydrochloric acid solution would separate inorganic phosphorus from a mixture of lecithin and starch. This mixture was used in order to approach the condition of the dry egg. The lecithin was dissolved in ether to get it in a fine state of division so as to be able to get a thorough mixture with the starch. The ether was driven off by warming on a water bath. To this mixture was added a known amount of sodium phosphate. The .2 per cent. hydrochloric acid solution

1. Hammerstein.

was added and was shaken at frequent intervals for three hours, then filtered. Suction was required as there was an emulsion formed when treated with the acid solutions. To an aliquot portion of this just enough ammonium hydroxide was added to make it basic to litmus paper, magnesium mixture was added without adding the 12 cc. of ammonium hydroxide, Sp. Gr. 90. This was then allowed to stand over night, then filtered and washed with 95 per cent. alcohol until free from ammonia. The filter paper and contents were dried, transferred to an Erlenmeyer containing 100 cc. of .2 per cent. nitric acid alcohol solution. After standing some time, filter, and take 75 cc. of this filtrate, evaporate nearly to dryness, take up with water and a little nitric acid, then precipitate with ammonium molybdate and reprecipitate with the magnesium mixture, igniting and finally weighing as the magnesium pyrophosphate. Table No. Y will show the complete recovery of the sodium phosphate by the method just given.

As the inorganic phosphorus is probably in the form of calcium phosphate a sample of pure calcium phosphate was digested and treated as in the determination of inorganic phosphate. It was found that the .2 per cent. hydrochloric acid would dissolve the calcium phosphate and on analysis gave identical results with sample digested with concentrated nitric acid and run by the official method.

TABLE Y

Laboratory Number	Na_2HPO_4 Equivalent in $Mg_2P_2O_7$	Added Lecithin	Recovered $Mg_2P_2O_7$
6 A	.0373	.6761	.0372
6 B	.0373	.6761	.0371
8 A	.0378	.7749	.0377
8 B	.0378	.7749	.0378
9 A	.0373	.5996	
9 B	.0373	.5996	.0378

The next step was the mixing of Ca3(P04)2, lecithin and starch to determine whether all of the phosphorus could be obtained, if so, there seems to be no doubt that the inorganic phosphate in the egg would be all dissolved by using the .2 per cent. hydrochloric extracting solution, as the phosphorus would be in a much finer state of division than the calcium phosphate worked with. Table W will show the results of a series of analyses.

TABLE W

Laboratory Number	Lecithin Added	$Ca_3(PO_4)_2$ Added	Weight of $Mg_2P_2O_7$ Found	Equivelent of $Mg_2P_2O_7$ Added
11 A	.5826	.1	Lost	.0688+
11 B	.5826	.1	.0688	.0688+
12 A	.4044	.1	.0689	.0688+
12 B	.4044	.1	.0689	.0688+
13 A	.3580	.1	.0687	.0688+
13 B	.3580	.1	.0686	.0689+
14 A	.3346	.1	.0688	.0689+
14 B	.3346	.1	.0694	.0688+

From this table it will be seen that all of the phosphorus may be determined by this method and that the lecithin will not be broken up. The method used in these determinations is the one adopted for the separation of the inorganic from the organic in the analysis of the dried egg. It is as follows:

Take 10 grams of the finely powdered substance, extract for three hours with 300 cc. .2 per cent. hydrochloric acid solution. Take an aliquot of this solution after filtering make just basic, then add 15 cc. of magnesium mixture, allow to stand over night, filter through a double filter and wash with 95 per cent. alcohol until free from ammonia, dry the paper and contents, transfer to an Erlenmeyer and digest with 100 cc. of .2 per cent. nitric acid alcohol solution, filter and take 75 cc. of this soluion, evaporate nearly to dryness, take up with water and a little nitric acid. Filter if necessary. Precipitate with ammonium molybdate and again with the magnesium mixture, finally ignite and weigh as the Mg2P207. This is the method used in obtaining all the results on egg analysis.

The inorganic phosphorus to be fed to the chickens outside of what is contained in the grain will be in the form of meat scrap or finely ground bone. The following table shows the percentage of phosphorous as inorganic, total, the organic being taken as the difference. The total phosphorus is as *P*, not the anhydride. In order to get a uniform sample of the meat scrap and also the ground bone it was necessary to extract with ether in order to remove the fat so as to grind the sample. The per cent. of phosphorus was determined on the original basis. The cabbage as shown in the analysis was to be fed as a green food but was withdrawn from the ration.

The feed given to the one set of chickens will be called organic ration; the other, the inorganic ration. The organic ration comprises buckwheat 1, wheat 1, pea meal 1-2, cubical grit or ground

oyster shell 1, the nutritive ratio of this 1:64. In order to get some term to show the proportion of organic and inorganic phosphorus, we will call this ratio the phosphorus ratio, the ratio in the feed called the organic feed is 1:17. The inorganic phosphorus feed, which is made up of corn 1, oats 1, wheat (treated) 1, bone meal 1. The nutritive ratio is 7:5.6, while the phosphorus ratio is 1:30. It will be seen that there is a wide varying ratio between the two forms of feeding stuffs. The wheat termed "treated wheat" was treated as follows:

The whole wheat soaked for twenty-four hours in water at ordinary temperature, as there seems to be no difference as to the amount of organic phosphorus taken from the wheat when treated hot and cold water. The wheat after this treatment is drained for half an hour and fed to the chickens, or a large amount treated in this manner, thoroughly dried and kept for further feeding. This treatment removes some of the organic phosphorus in the form of Phytin. The amount removed is comparatively small as may be seen in Table A.

TABLE Z

Laboratory Number	Total Phosphorus	Inorganic Phosphorus	Organic Phosphorus by Diff.	Name and Remarks
378	.328	.031	.296	Cornmeal.
379	.360	.023	.337	Ground wheat.
381	.348	.031	.297	Pin-head oatmeal.
382	.322	.....	.....	Corn.
383	.321	.....	.....	Wheat.
385	.513	.....	.....	Pea meal.
390	.272	.....	.....	Early sunflower.
384	.874	.016	.858	Buckwheat.
409	.864	.042	.822	Buckwheat middlings.
391	.340	.....	.....	Late sunflower.
407	3.32	3.49	.83	Meat scrap.
408	5.05	4.59	.46	Green cut bone.
419	.031	.007	.024	Cabbage—89 .20% H_2O.
55	.340	.0262	.378	Treated wheat—3-22-11.
56	.351	.026	.325	Treated wheat received April 6.

The condition of the chickens before the ration feeding was normal; they were laying well considering that they were in small pens, also housed. The chickens were fed on a uniform feed for several weeks before changing to the organic and inorganic rations in order to get them on a uniform basis.

The temperature at which the eggs must be dried was found to be a most important factor: when it comes to the determination of the

inorganic. Two samples of the same set of eggs (Pen 1) were taken, one portion was dried in a water oven at the boiling point of water, another portion was dried at between 45 and 50 degreesC. with a current of air blowing into the oven. It was found that the portion dried at the boiling point of water, gave a much higher percentage of *P* than the one dried at the lower temperature. This made it necessary to find the temperature at which the drying must be carried on, so as not to change the organic phosphorus into an available form to be extracted by the .2 per cent. hydrochloric acid. One drying was carried on in a vacuum oven water jacketed, in one end was placed a glass tube drawn out to a very fine point; this was broken off in order to allow air to leak in very slowly thus giving a current of air to carry out the moisture. The oven as described would maintain a column of mercury approximately seventeen inches.

TABLE A

Temperature °C	Total Phosphorus	Inorganic Phosphorus	Remarks
95—100	.829	.08	Pen 1.
45— 55	.85	Trace	Pen 1.
65— 67	.83	.016	
75— 77	.84	.013	
95—100	.87	.049	
46	.78	.015	Leaky Vacuo.

The temperature at which the eggs were dried, the analysis of which will be seen in the various tables is between 47 degrees and 50 degrees, using water ovens and having a current of air blowing into the oven. This gives a rapid, thorough drying if the eggs are kept well stirred. The analysis of the eggs before the feeding of the organic and inorganic rations may be seen in Table 1. This serves as a basis of comparison for the experiment tables of analysis.

TABLE I.

Pen Number	Laboratory Number	Total Weight of Eggs	Weight of Yolks and Whites	Weight Dried	Total Phosphorus %	Inorganic Phosphorus %	Organic Phosphorus %
26 A	9	223.8	197.8	54.5	.798	.009	.789
26 B	10	62.9	56.1	14.4	.80	.014	.786
26 C	11	58.2	52.2	14.2	.826	.017	.809
26 D	12	215.	180.	54.3	.788	.012	.776

The feed given the fowls during the time of this feeding was grain, shell, grit, meat scrap and some mash from time to time in order to induce them to lay. The feed consumed by the chicken during the expirement may be seen in the tables to follow. The tables are the weekly consumption of food while the analysis are for four day periods at first with a lengthening of the periods towards the end of the experiment.

Pounds of Feed Consumed, Week Ending March 20, 1911

TABLE II.

Pen	Grain	Shell	Grit	Pea Meal	Bone Meal	Green Bone
26 A	5.9	.7	.3	.8		
26 B	5.8	1.1	.2	1.5		
26 C	5.7		1.5		.5	1.6
26 D	5.2		.3		.5	1.6

First Period of Feeding the Organic and Inorganic Rations
March 15-19, 1911

TABLE III.

Pen Number and Date	Laboratory Number	Weight of Eggs	Weight of Yolks and Whites	Weight Dried	Total Phosphorus %	Inorganic Phosphorus %	Organic Phosphorus %
26 A March 16, 17, 18	15	164.5	143.6	40.4	.796	.01	.786
26 B March 16, 16, 18, 18	16	217.	189.8	53.7	.76	.008	.754
26 C March 16, 17, 17	17	169.	140.8	42.1	.818	.009	.809
26 D. March 16, 16, 18, 18	18	219.5	194.5	62.3	..82	.01	.81

Shells from A and B .133% total phosphorus.
Shells from C and D .139% total phosphorus.

Pounds of Feed Consumed, Week Ending March 27, 1911

TABLE IV.

Pen	Grain	Shell	Grit	Pea Meal	Bone Meal	Green Bone
26 A	4.4	.6	.2	.6		
26 B	4.8	4.5	.1	1.7		
26 C	5.6		4.6		.0	1.9
26 D	6.0		1.4		.3	1.8

Second Period of Feeding the Organic and Inorganic Rations March 19-22, 1911

TABLE V.

Pen Number and Date	Laboratory Number	Weight of Eggs	Weight of Yolks and Whites	Weight Dried	Total Phosphorus %	Inorganic Phosphorus %	Organic Phos. phorus %
26 A March 20, 21, 22.....	19	157.8	136.5	34.6	.77	.007	.763
26 B March 19, 21.....	20	119.7	104.7	30.8	.774	.01	.763
26 C March 19, 20, 31, 22	21	214.5	190.	53.	.81	.012	.798
26 D March 20, 20, 22.....	22	178.7	157.6	42 5	.91	.016	.793

Shells from A and B .12% total phosphorus.
Shells from C and D .105% total phosphorus.

Pounds of Feed Consumed, Week Ending April 3, 1911

TABLE VI.

Pen	Grain	Shell	Grit	Pea Meal	Bone Meal	Green Bone
26 A	4.6	.4	.4	.5		
26 B	5.1	.3	.1	1.9		
26 C	4.6		.8		.1	1.8
26 D	4.3		1.9		.4	1.8

Third Period of Feeding the Organic and Inorganic Rations
March 23-27, 1911

TABLE VII.

Pen Number and Date	Laboratory Number	Weight of Eggs	Weight of Yolks and Whites	Weight Dried	Total Phosphorus %	Inorganic Phosphorus %	Organic Phosphorus %
26 A March 23, 23, 23	25	153.2	132.	34.5	.771	.008	.763
26 B March 24, 24, 26	25	164.4	143.2	36.4	.779	.011	.768
26 C March 23, 23, 24	27	158.7	141.9	37.8	.815	.013	.802
26 D March 24, 25, 26	28	175.7	153.5	42.9	.81	.01	80

Shells from A and B .133% total phosphorus.
Shells from C and D .132% total phosphorus.

Pounds of Feed Consumed, Week Ending April 10, 1911

TABLE VIII.

Pen	Grain	Shell	Grit	Pea Meal	Bone Meal	Green Bone
26 A	4.0	.6	1.0	.7		
26 B	4.9	0.0	.2	.8		
26 C	5.6		.1		.3	1.8
26 D	5.4		.0		.5	1.8

Fourth Period of Feeding the Organic and Inorganic Rations
March 27-31, 1911

TABLE IX.

Pen Number and Date	Laboratory Number	Weight of Eggs	Weight of Yolks and Whites	Weight Dried	Total Phosphorus %	Inorganic Phosphorus %	Organic Phosphorus %
26 A March 27, 29, 30.....	31	178.8	155.	43.	.78	.011	.779
26 B March 27, 29, 29	32	169.7	148.	40.6	.79	.01	.78
26 C March 27, 28, 30	33	169.7	151.9	61.6	.823	.013	.81
26 D March 27, 28, 29, 30	34	216.5	192.9	92.6	.827	.012	.815

Shells from A and B .13% total phosphorus.
Shells from C and D .145% total phosphorus.

Pounds of Feed Consumed, Week Ending April 17, 1911

TABLE X.

Pen	Grain	Shell	Grit	Pea Meal	Bone Meal	Green Bone
26 A	37	3.2	3.1	1.1		
26 B	38	4.4	.6	.6		
26 C	39	4.6			.5	1.8
26 D	50	4.8			.9	1.8

Fifth Period of Feeding the Organic and Inorganic Rations
March 31 to April 6, 1911

TABLE XI.

Pen Number and Date	Laboratory Number	Weight of Eggs	Weight of Yolks and Whites	Weight Dried	Total Phosphorus %	Inorganic Phosphorus %	Organic Phosphorus %
26 A March 31 – April 1, 2, 3, 4, 5	37	337.	292.	80.	.782	.01	.772
26 B March 31 – April 1, 2, 3, 4, 5	38	337.	292.	7.77	.78	.007	.773
26 C March 31 – April 1, 2, 3, 4, 5	39	119.5	103.5	27.5	.829	.013	.816
26 D March 31 – April 1, 2, 3, 4, 5	40	358.	314.5	87.7	.846	.015	.831

Shells from A and B .133% total phosphorus.
Shells from C and D .125% total phosphorus.

Pounds of Feed Consumed, Week Ending April 24, 1911

TABLE XII.

Pen	Grain	Shell	Grit	Pea Meal	Bone Meal	Green Bone
26 A	2.4	.0	.0	2.1		
26 B	3.5	22	.1	2.1		
26 C	4.1		.3		.9	1.1
26 D	4.2		.3		1.1	1.1

Sixth Period of Feeding the Organic and Inorganic Rations
April 6-12, 1911

TABLE XIII.

Pen Number and Date	Laboratory Number	Weight of Eggs	Weight of Yolks and Whites	Weight Dried	Total Phosphorus %	Inorganic Phosphorus %	Organic Phosphorus %
26 A April 7, 8, 9, 10, 11	43	275.9	239.7	65.7	.79	.019	.781
26 B April 7, 10	44	98.9	85.6	24.5	.78	.01	.77
26 C April 6, 7, 8, 9, 10, 11	45	321.2	284.8	77.5	.833	.012	.821
26 D April 6, 7, 9, 11.....	46	237.6	208.	58.2	.829	.014	.815

Shells from A and B .128% total phosphorus.
Shells from C and D .129% total phosphorus.

Pounds of Feed Consumed, Week Ending May 1, 1911

TABLE XIV.

Pen	Grain	Shell	Grit	Pea Meal	Bone Meal	Green Bone
26	2.8	.0	0.0	.6		
26	3.9	.5	.1	1.0		
26	4.0		0.0		.7	1.5
	5.1		.3		.6	1.5

Seventh Period of Feeding the Organic and Inorganic Rations to April 24, 1911

TABLE XV.

Pen Number and Date	Laboratory Number	Weight of Eggs	Weight of Yolks and Whites	Weight Dried	Total Phosphorus %	Inorganic Phosphorus %	Organic Phosphorus %
26 A April 22, 23	49	109.2	95.9	24.5	.796	.009	.787
26 B April 23, 23, 23.....	50	161.5	140.8	38.	.775	.0.	.764
26 C April 22, 22	5*	115.	132.5	34.5	.841	.013	.848
26 D No eggs received from this pen during this period.							

Shells from A and B .128% total phosphorus.
Shells from C and D .136% total phosphorus.

The following table will show the weight of the individual hens before the penning and after the experiment, Pen A having lost two fowls during the feeding.

Pen	Before	After	Loss or Gain
A 1	3.17	2.95	—0.22
2	3.67	3.05	—0.62
3	3.94	2.90	—I.04
B 1	3.65	3.50	—0.15
2	3.15	2.50	—0.65
3	3.51	2.90	—0.61
4	3.58	2.85	—0.73
5	3.70	2.75	—0.95
C 1	3.44	3.15	—0.29
2	4.04	3.70	—0.34
3	2.61	3.00	+0.39
4	3.46	3.65	+0.19
5	3.36	2.90	—0.46
D 1	3.56	3.20	—0.36
2	3.68	3.50	—0.18
3	4.12	3.75	—0.37
4	4.33	4.80	+0.47
5	3.85	3.70	—0.15

The increase of the organic phosphorus content of the eggs from Pen C and D would indicate that there should be an increase in the

lecithin content. To see whether this is true, two samples of No. 40 and No. 38 were taken, extracted with chloroform for twenty hours, evaporated to dryness and total phosphorus determined in the usual manner.

40 A..	.3% P.
40 B.	.29% P.
38 A.	Lost.
38 B.	.22% P.

If these were converted into lecithin by multiplying by the factor 7,2703, 40a would give 7.92 per cent. lecithin, while 38b would give 5.82 per cent. lecithin.

Conclusion

The data seems to indicate that the fowls fed on a high inorganic ration have increased their organic phosphorus content.

That the increase of the organic content shows an increase in the lecithin.

The weight of the shells remains practically constant.

The inorganic phosphorus content remains practically constant.

The phosphorus content of the egg shell does not increase.

This indicates that the excess inorganic phosphorus fed to the fowls passes out of the body.